



# NANOPARTICLE BASED TECHNOLOGY

Nanotools for Life Sciences and  
Pharma Industries



Customized service



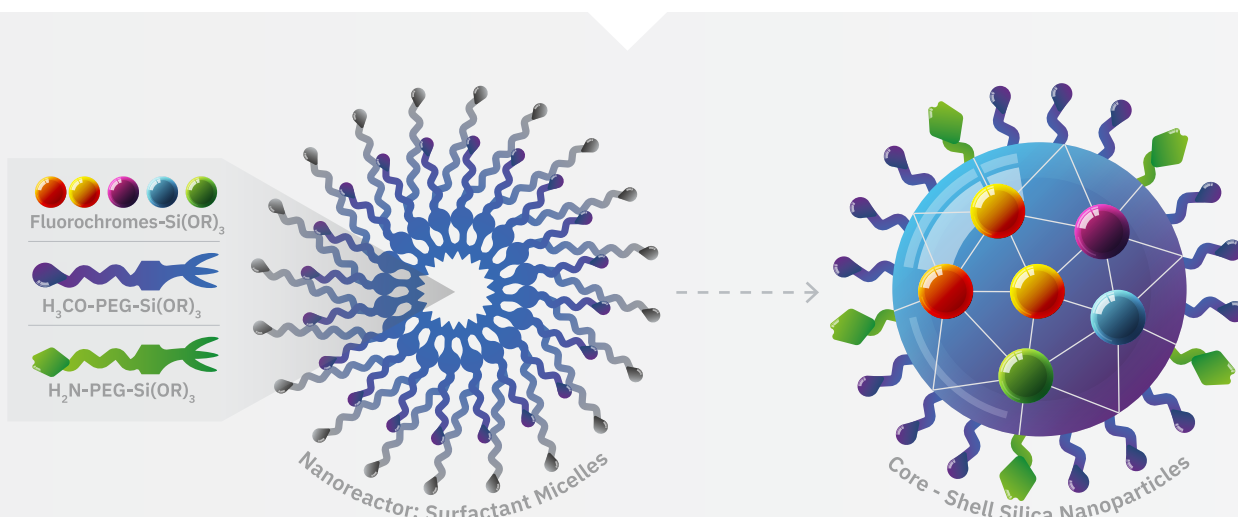
Competence & Versatility  
make AcZon a partner  
rather than a supplier.

# WELCOME TO THE NANOMETRIC WORLD

## How the infinitely small revolutionizes the scientific world

### AcZon proprietary core-shell silica nanoparticles (SiNPs) represent a novel class of probes for biological applications.

SiNPs are spherical and their diameter varies between 10 and 70 nm; they are made up of two structural compartments: the core and the shell. During the synthetic procedure, the chemical-physical characteristics of the SiNPs can be personalized according to the intended use. They can be modified, both internally (core) and externally (shell) in step with customer requirements.



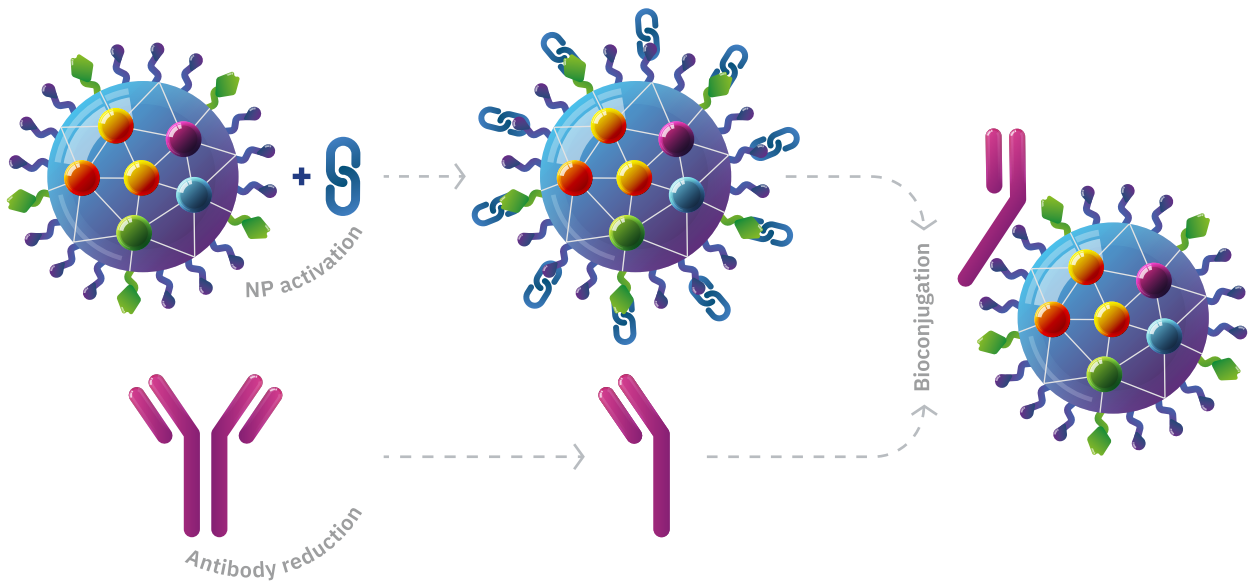
AcZon has put great efforts, in recent years, into the development of its patented line of nanoparticle-based products. AcZon nanoparticle synthesis is a micelle assisted method in which all the reagents spontaneously arrange in a surfactant micelle. Thanks to the addition of a catalyst and a silane precursor, the reaction takes place to form fluorescent nanoparticles.

The nanoparticle itself acts as a shield which protects the molecules hosted in the inner part.

As a result, all the molecules included in the nanoparticle core are not affected by external agents as pH and even by continuous light irradiation.

Thanks to the spherical shape of nanoparticles and the consequent elevated surface/volume ratio, more active molecules (such as enzymes, drugs, chelating agents, etc) can be conjugated to a single targeting molecule assuring a consistent effect enhancement.

THE SHIELD EFFECT

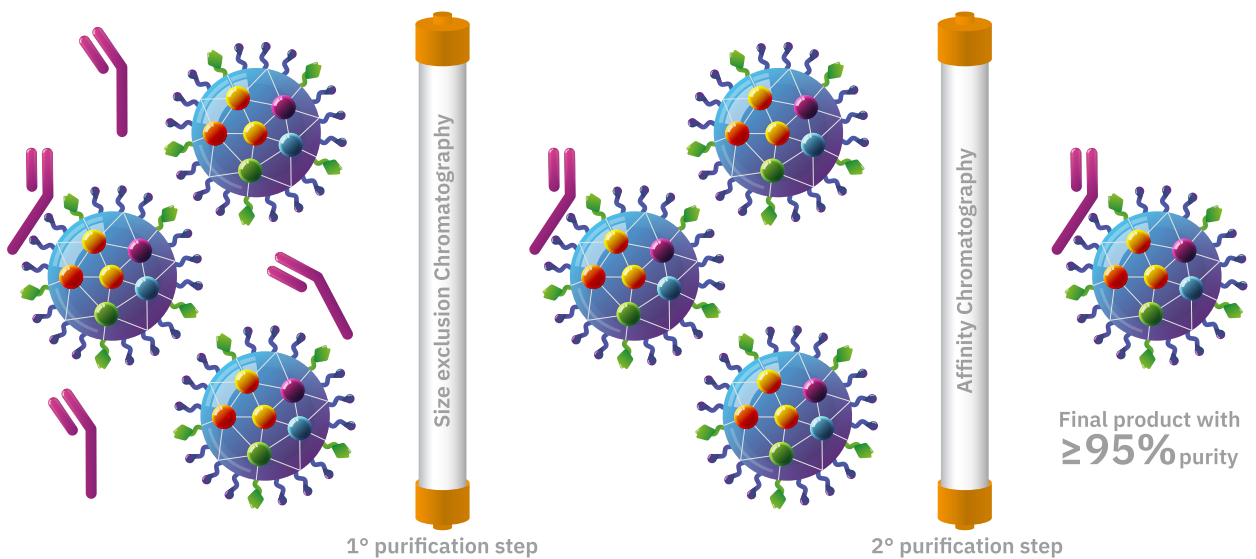


The shield effect exerted by nanoparticle, allows the application of a revolutionary purification protocol of bioconjugates assuring a higher purity degree of the final conjugates with consequent reduced background signals. In brief, the protection offered to the sensitive dyes included in the nanoparticles, allows the adoption of an affinity chromatography

purification step which requires a low pH elution otherwise impossible to apply due to the intrinsic sensitivity of conventional used probes.

The coupling of the historically used purification method by size exclusion chromatography to the second affinity step increases the purity degree of the final conjugated  $\geq 95\%$ .

DOUBLE PURIFICATION STEP



The protection offered by the silica matrix against low pH allows an additional purification step on affinity chromatography resulting in a final purity higher than 95% increasing, once more, the signal/background ratio.



# SILICA NANOPARTICLES VERSATILITY AND APPLICATIONS

Thanks to the possibility to synthesize various kind of nanoparticles (amongst others silica, magnetic, polymeric) and the ability to control their size, to modify surface characteristics and conjugate them with other species as antibodies, oligonucleotides and drugs (just to name a few), AcZon's patented Silica NanoParticles are considered a dramatically versatile tool which allows a multitude of applications.

The combination of nanotechnologies and medicine radically increases the possibilities in both life sciences and healthcare providing, day by day, new and surprising solutions.

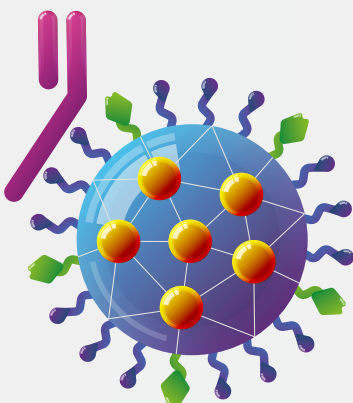
## Cytometry and immunofluorescence

Stability, intensity and clarity of signal are always issues limiting the application of flow cytometry and fluorescence microscopy. Thanks to the inclusion and modulation of dyes in the nanoparticle matrix, AcZon finally solved these problems.

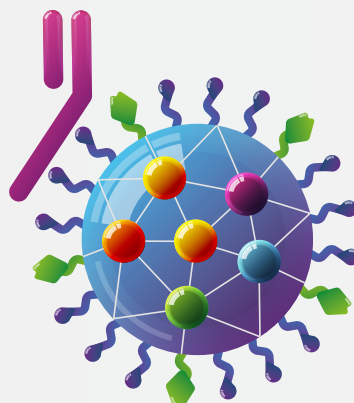
## Cytometry and immunofluorescence

To enlarge the Stokes shift and overcome all the drawbacks typical of tandem dyes used in flow cytometry, AcZon offers silica nanoparticles which host up to 6 fluorescent dyes which exploit FRET phenomenon.

NanoChromes



NanoTandems



## Research and innovation: The basis of AcZon DNA

AcZon is an Italian biotech company with an experienced team of researchers (chemists and biologists) with a strong expertise in the field of nanotechnology and bioconjugations.

### Immunoassays in QC labs

The signal clarity and the high number of plate failures are well known issues of immunoassays.

AcZon developed silica nanoparticles with a dye in the core and, on the shell, a higher and adaptable number of detection enzymes (HRP o AP) and superior quality secondary antibodies to dramatically reduce plate failures in both ELISA and LISA-F.

### Imaging

One of the most relevant drawbacks of in vivo diagnostics is the unavailability of reagents which enable for both in vivo and ex vivo analyses.

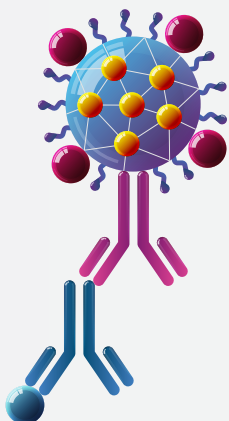
AcZon offers an innovative nanoparticle-based bimodal tool with targeting molecules and chelating agents (to bind radionuclides) on the shell and a high number of fluorescent molecules in the core. With this revolutionary tool is made possible to perform in vivo analyses by  $\mu$ PET and, after animal sacrifice to study explanted tissues with fluorescence microscopy.

### Drug delivery

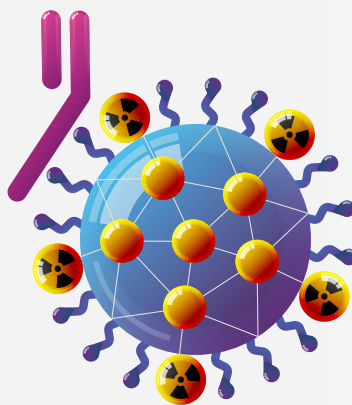
Nowadays, the trend in cancer therapy is to directly deliver the drugs on target cells.

AcZon designed a trimodal tool where dyes and drugs are included in the silica matrix and the shell is functionalized with targeting and functional molecules. This pioneer reagent allows the contemporary delivery of the drug on the site of action of target cells and the monitoring of the fate of the tool itself.

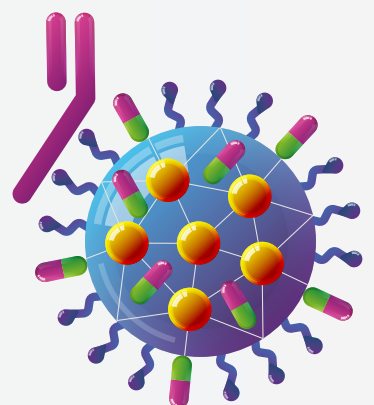
**NanoLISA & NanoLISA-F**



**NanoRad**



**NanoCarrier**



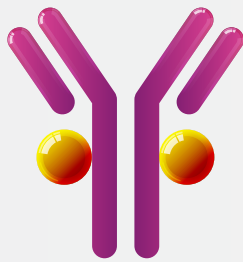
# IMPROVE THE QUALITY OF YOUR FLOW CYTOMETRY ASSAY

## The new era of tandem dyes

Since the earliest application of flow cytometry to the study of cells, there has been a drive to increase the number of distinct measurements for each cell achieved by the development of new fluorophores as tandem dyes. The tandem dyes are highly sensitive and degrade easily.

In AcZon proprietary NanoTandems, this issue has been fixed thanks to the presence of the silica matrix acting as a shield protecting these sensitive molecules from the degradation allowing to maintain the same instrument setting for the whole reagent validity.

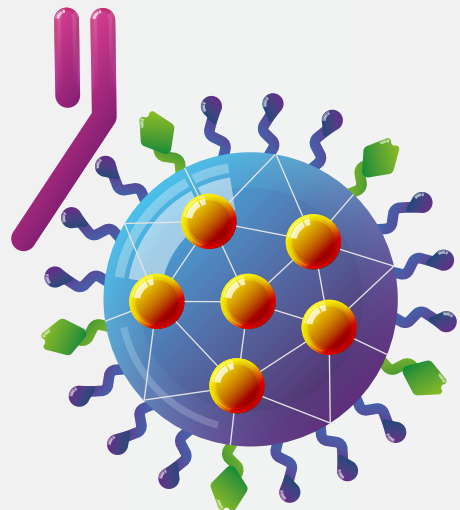
### Flow cytometry common reagents



**The most notable limitation of tandem dyes is their high susceptibility to degradation.**

In conventional antibody-dye conjugates the sensitive fluorescent molecules are directly in contact with the external environment and susceptible to its effect. In addition, the number of dyes per antibody is limited to the reactive groups available on the protein.

### NanoChromes & NanoTandem



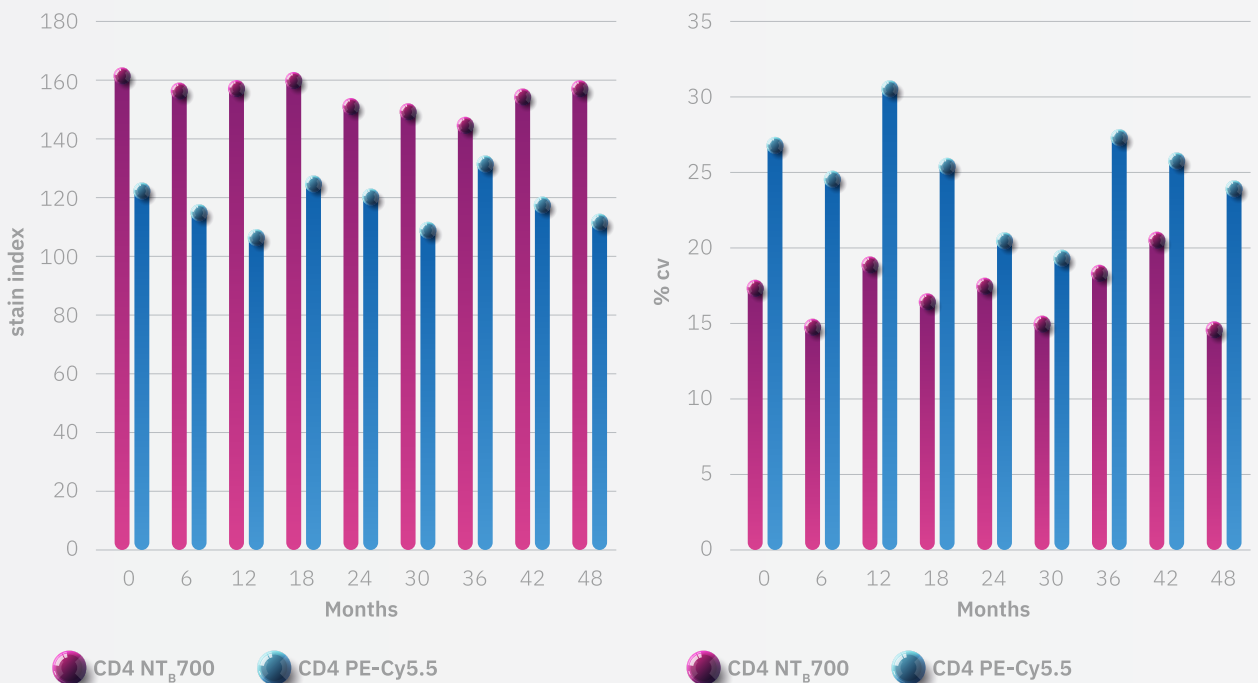
AcZon nanoparticle-based dyes allow for the conjugation of a higher number of fluorescent molecules per anchoring point on the protein with a significant increase in the signal. The confinement of sensitive dyes in the matrix confers unique properties to the reagents.



In addition, is not infrequent to obtain false negative and false positive results caused, respectively, by the low expression of antigens and by the non-specific binding of the probe to cells not expressing the antigen of interest.

The nanoparticles, hallmark of **NanoChromes** and **NanoTandem**, solve these problems concentrating and insulating fluorescent dyes inside the silica matrix allowing a higher number of dyes per antibody and, contemporary, avoiding their non-specific binding.

Moreover, the protection offered by the matrix allows the further purification step which decreases the level of impurity resulting in a higher signal/background ratio.

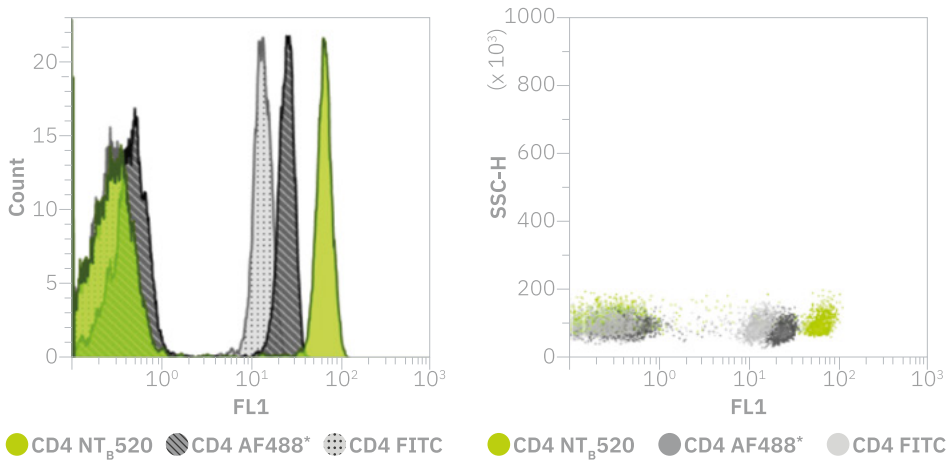


The reproducibility offered by NanoTandems is highly superior to the one offered by conventional fluorophores. To gather the data above reported 3 different competitor lots have been employed due to their short shelf-life. In addition, the instrument setting was changed 5 times with the conventional reagent because of the increasing contribution of RPE due to Cy5.5 damage. No change after the preliminary setting was operated using AcZon reagent.

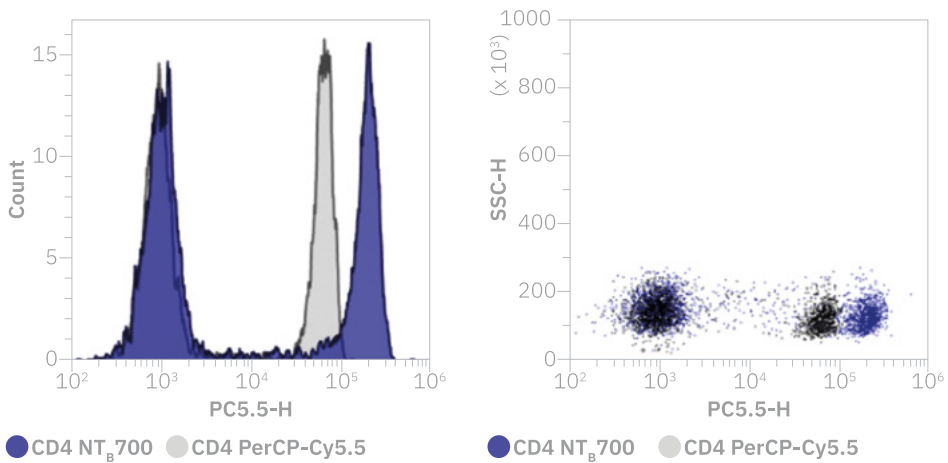
## >FLOW CYTOMETRY IN RD ACTIVITIES

In the past, flow cytometry has been underutilized as a screening tool due to the restrictions in handling large numbers of samples.

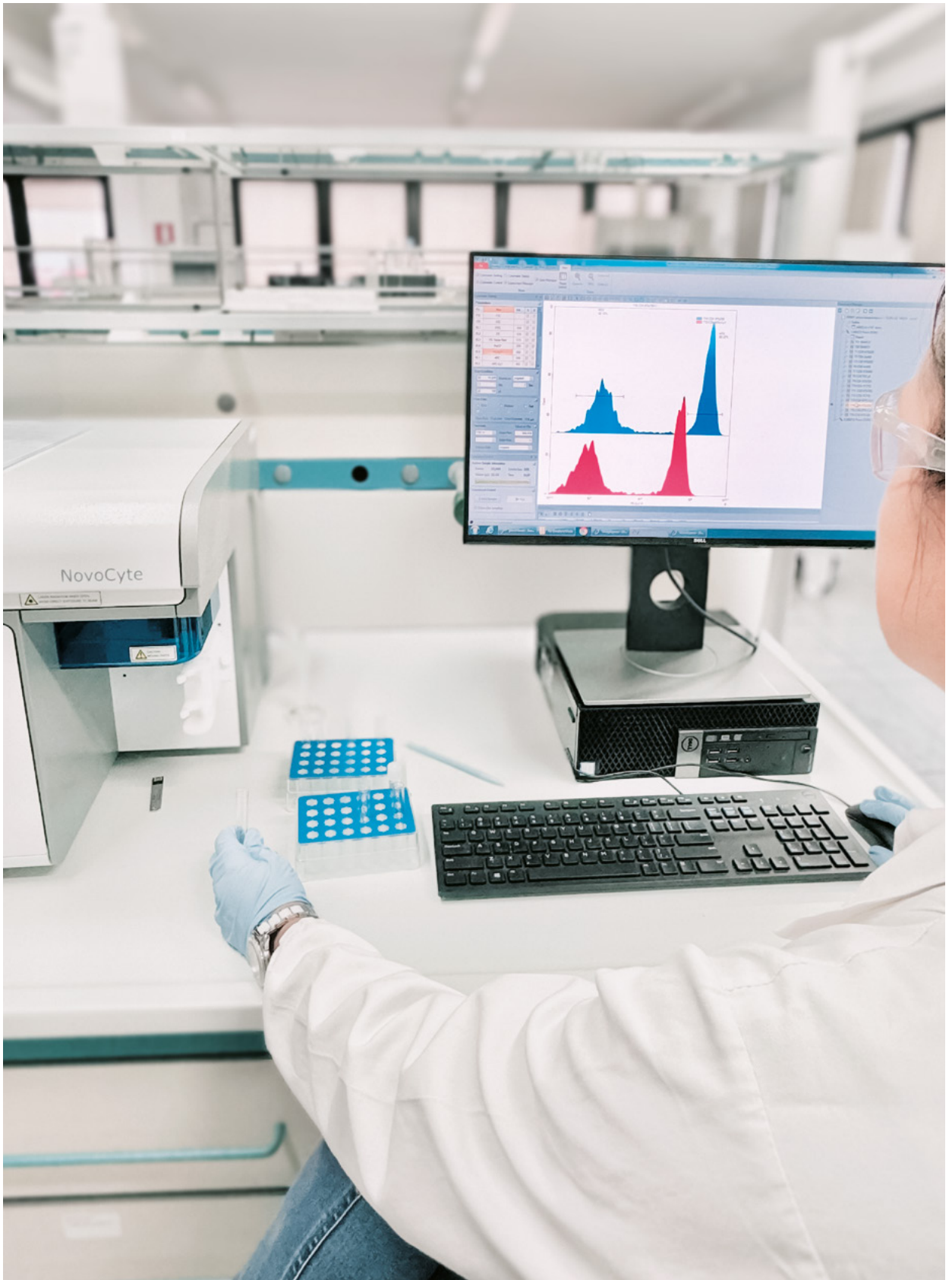
Recently, with the advent of new technologies for the development of both instruments and reagents, it is gaining more and more importance in the research and development projects of pharmaceutical and biotech industries.



\*Alexa Fluor is a registered trademark of Life Technologies Corporation.



Mouse anti Human CD4 (clone EDU-2) on peripheral whole blood from healthy donor. A comparison between AcZon nano-based reagents and the golden standards.



Flow cytometry quality control station: AcZon's standard products are sold in ready-to-use format where 10  $\mu$ l are sufficient to label  $0.1-1 \times 10^6$  leukocytes in 100  $\mu$ l.

In the picture the comparative assessment between AcZon Ms anti Hu CD4 (EDU-2) labelled with NT<sub>b</sub>780 (light blue) and PE-Cy7 (red) on healthy donor peripheral blood.

# DESIGNED TO REDUCE ASSAY FAILURES

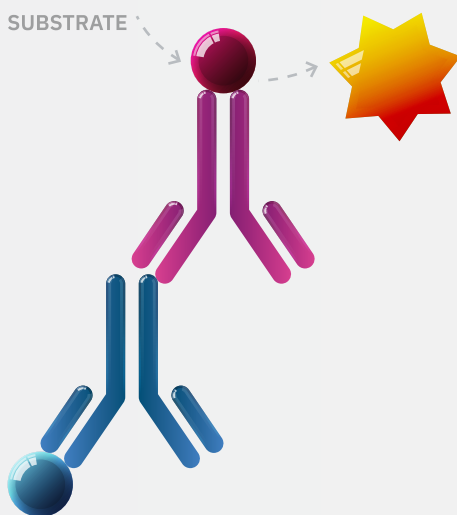
## AcZon gives the solution

Plate failure is one of the main issues to be faced from those laboratories working with high number of ELISA immunoassays such as quality control departments of pharma companies.

**The reasons behind this important loss of time and money include the high percentage of false positive and negative results and the well-known reagent instability which requires frequent lot concentration assessments.**

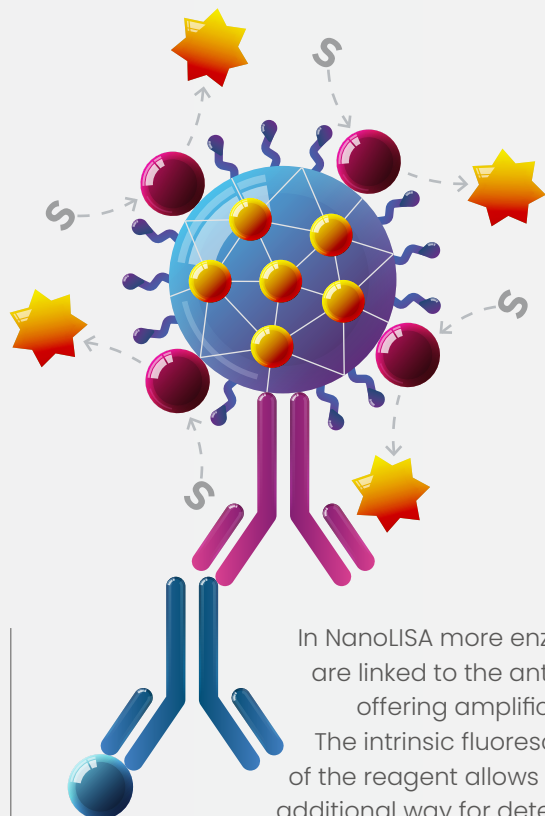
AcZon gives the solution to these problems offering the new proprietary product line NanoLISA & NanoLISA-F for the indirect detection by colorimetric reaction and fluorescence. Thanks to the high number of revelation molecules (enzymes or dyes) they allow the detection of low concentration of antigens. In addition, the high purity of the raw materials employed assures the stability of the reagent until the expiry date without need of additional titrations.

ELISA common reagents



In conventional reagents a few enzymes can be conjugated to the antibody. Consequently, the detection reaction results in a smaller quantity of reacted material.

NanoLISA & NanoLISA-F

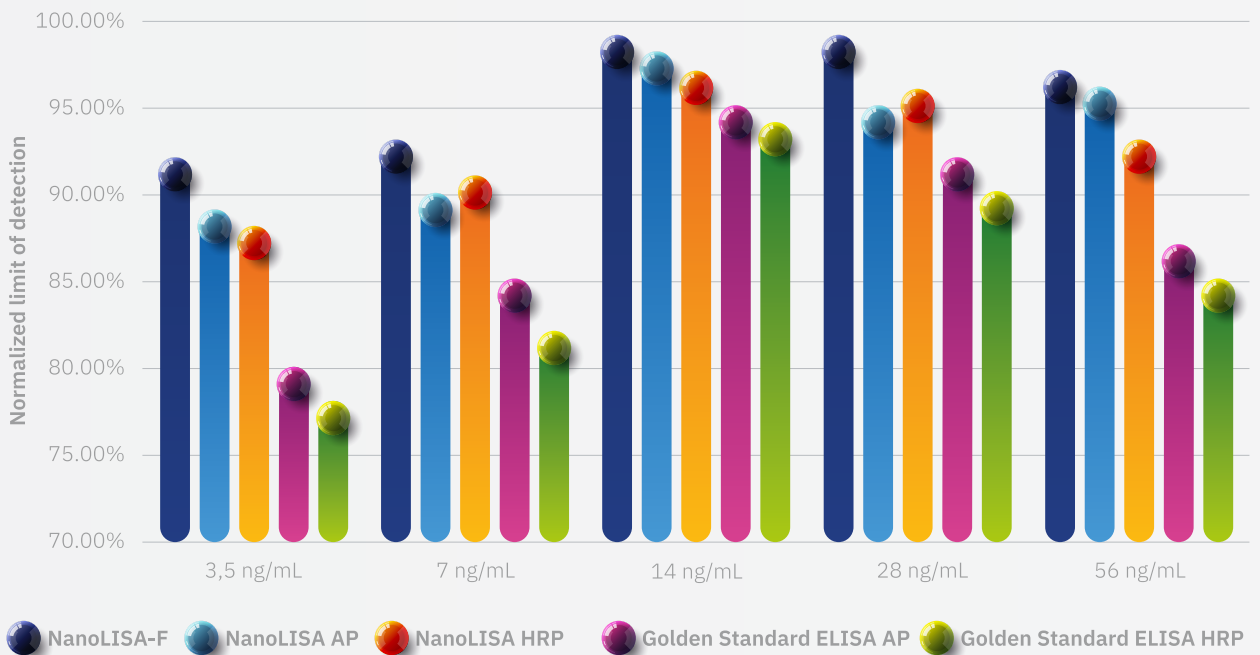


In NanoLISA more enzymes are linked to the antibody offering amplification: The intrinsic fluorescence of the reagent allows for an additional way for detection.

## >ELISA TESTS IN QC ACTIVITIES

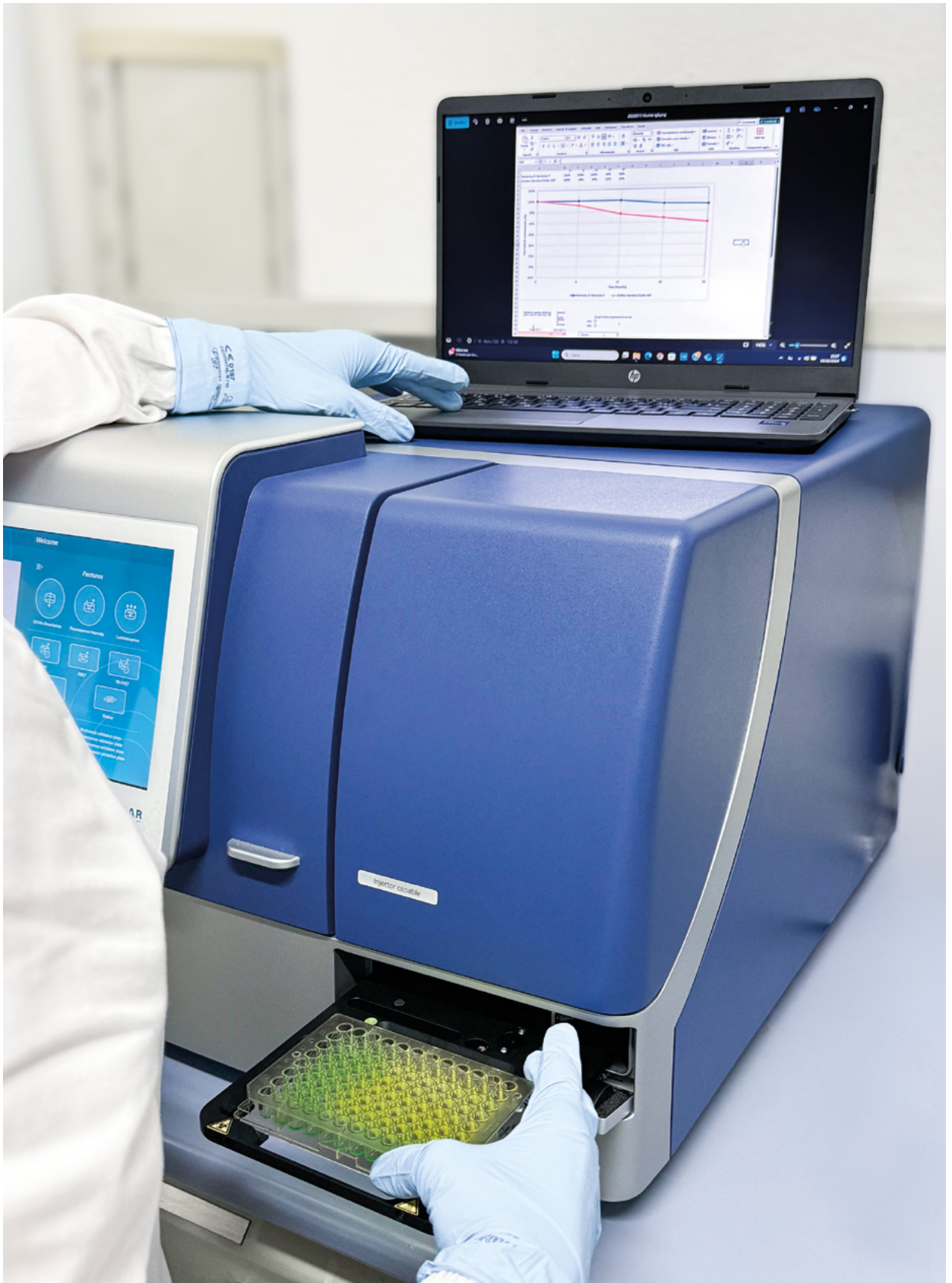
Failure in analyte quantification is one of the most common bumps in the road in pharma quality control.

Thanks to AcZon attention to the quality of raw materials, semifinished products and the strict control operated on the production process, the reliability and the quality of the whole range of products is higher and constant across the whole validity of the lot.



Human IL-6 was spiked at different concentrations in human plasma samples for ELISA detection. The primary antibody concentration was maintained constant among the cases. The chart represents the overall recovery in the spiked samples (average of 3 independent experiments).





Colorimetric test quality control station: the control of AcZon's products from NanoLISA & NanoLISA-F line is carried on using commercial golden standards as reference.

In the picture mock samples (murine IgGs spiked in complete cellular medium at different concentrations) were detected using NanoLISA & NanoLISA-F in its HRP version, and the competitor counterpart.



Quality is not act.  
It is a habit.

Aristotle

# ACZON'S LONG-TIME EXPERIENCE AT CUSTOMER DISPOSAL

## Not only nanoparticle-based reagents

The production process of conjugate antibodies and reagents is entirely carried on in Monte San Pietro headquarter offering wide margin of customization.

**AcZon is glad to make the whole company's know how available for customers' needs.**

For this reason, AcZon put in place a custom-made department ready to accommodate the requests that go beyond the product lists. It would be simplistic to limit these services to a list, but AcZon custom-made services include the custom conjugations of customer's proteins, the personalization of product formulation (buffer, concentration, additive, ...), the optimization of experimental protocols, just to name a few.

**AcZon's goal is to be considered an extension of customers' laboratories.**

## Our support to cut money and time losses

The average cost of developing and getting on the market a new drug is estimated in more than a billion of US dollars. In a such competitive market and environment, it is mandatory to avoid losing time and to proceed straightforward to the goal.

**AcZon development and research team is well aware of these needs and is highly committed to offering a complete service to relieve industrial researchers from troubles other than those deriving from science and not from suppliers.**





The protective effect given by the silica matrix towards sensitive dyes, permits a double purification assuring a higher purity of the final conjugate.



We work, day by day,  
to make researchers'  
activities more  
successful.

AcZon  
as important piece  
of research projects

Efficient knowledge sharing is a fundamental support for the innovation and development of research teams.

For this reason, **AcZon looks forwards to participating in both national and international projects with the aim to contribute to innovation offering knowledge, technology and a cutting-edge facility.**

AcZon's facility and know how is fully available for customers.



## Our beliefs

Geraldine Richmond, an American chemist, says *"If you don't have failure, then you are not really studying the unknown"*.

Scientists understand that failure is around the corner and often is an opportunity of improvement, but there are different classes of mistakes.

**AcZon mission is to provide scientists with high quality reagents and services manufactured according to a certified quality management system to avoid the uncertainty due to poor supplies.**

AcZon team is composed of highly qualified and experienced chemists, biologists and biotechnologists which collaborate in a synergistic way towards the identification and the resolution of the main issues affecting researchers. In accordance with this feature, the production process is regulated by strict quality control check points which assure the performance of the final products. The know-how and the versatility of the whole team allow to customize each production step according to specific needs and requirements from the customer.

AcZon's custom made services are thought to personalize also (but not only) buffer, concentration and packaging of the products.

View the most up-to-date product lists on [www.aczonpharma.com](http://www.aczonpharma.com) and stay connected to discover more or directly contact us.





# ACZON: ITALIAN EXCELLENCE IN THE BIOTECH FIELD

## Research and innovation: The basis of AcZon DNA

AcZon is an Italian biotech company with an experienced team of researchers (chemists and biologists) with a strong expertise in the field of nanotechnology and bioconjugations.

**Thanks to the encounter between these important disciplines, AcZon developed a new line of products based on fluorescent silica nanoparticles.**





## Research

The research activities are, for AcZon, fundamental pillars for the continuous identification and development of new reagents featured by extended stability, amplified signals, decreased analysis time and reduced test failures.

## Manufacturing process

The AcZon production process takes advantage of cutting-edge equipment. The strong interconnection between automation and human competency, together with a perfect organization of processes and procedures with a decisive quality control department allow to offer high quality standards. Problem-solving attitude is an intrinsic feature of the team ensuring high grade tailor-made services.

## Sales

AcZon products are sold under the AcZon brand but also through OEM licenses. The technical commercial staff will place side by side the customers during the whole purchasing experience and in the post sales.

Don't forget that near to the innovative products from the nano world, AcZon offers a wide range of tools to accelerate your success including a panel of internally produced antibodies conjugated with conventional dyes.

Last but not least, AcZon technical support will be at your side with protocol additional details, experimental set-up or troubleshooting.



AcZon headquarter in Monte San Pietro (Bologna) where the development and production processes take place with strong interconnections.

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Be passionate about solving  
the problem, not proving  
your solution

Nathan Furr



AcZon is an Italian company with  
a legacy of constant research,  
innovation, and quality production  
processes for top-tier products.

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